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FINAL REPORT

RAPID METHODS FOR ISOLATION OF PATHOGENS AND THEIR TOXINS FROM FOODS

19940198

Funded by
The Agriculture Development Fund

January 2001
Prepared by: University of Saskatchewan

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19940198

FINAL REPORT- MAY 4, 2000

RAPID METHODS FOR ISOLATION OF PATHOGENS AND THEIR TOXINS FROM FOODS

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Historical note:

My capacity in this report came about because of my willingness to perform a collegial duty to help a fellow junior professor who was ill.

This grant proposal was written and submitted to SADF by Dr. Linda L. S. Yu of the Department of Applied Microbiology and Food Science to SADF in 1994-5. The Application generated a Contract signed between the two parties in the Fall of 1995. There were two graduate students of Dr. Yu working on aspects of this Contract and on whose M. Sc. Research Committee I was serving. Soon after Dr. Yu was diagnosed with breast cancer and underwent both surgery and chemotherapy. Her situation initially improved but subsequently worsened and her cancer spread leading to her final withdrawal from her position and leaving of Saskatoon. Because the students' research was underway, the Department Head then, Al McCurdy, requested that I oversee this situation, until there was some resolution to Dr. Yu's health. In the intervening years the students had finished their degree work and defended their thesis and generated useful data. Dr. Yu has opted out for permanent disability leave and her involvement with the University as of 1999 has been concluded.

I have brought my overseeing function to a conclusion. Here is the final research report with a review of options available today, as compared to 1995, when the original author made this application.

(a) Abstract/Summary

The intention of this proposal was to assess the presence of bacterial isolates associated with contamination of foods and foodborne pathogens include many traditional and modern tests of the year 1994-6. The classic mouse pathogenicity test, Anton's test, and toxicity test using chicken embryos are a few in the case of the bacterium, *Listeria monocytogenes* and there are equal numbers of tests if not many more in the case of *Escherichia coli*. Irrespective of their currency, these methods require expenditures in labor, capital and time. Thus, any one method's reliance on the type and capital expenditures of experimentation and result analysis for speed, and reliability is important. This project determined and has previously reported tests and their optimization for *E. coli* and *Listeria* strains and use anti-bacterial sera for specific discrimination from other food associated bacteria whether or not pathogenic.

(b) Executive Summary.

Many human pathogens including *Escherichia coli* and *Listeria monocytogenes* are food-borne and are responsible for serious infections and disease. These classical pathogens have recently become the subject of renewed interest because of acknowledged epidemics caused by food contaminated with *L. monocytogenes* and the now famous "hamburger disease" of the 1994-95 by *E. coli* O157:H7. Substantial interest and effort have been expanded recently in establishing new methods as well as improving classical ones for the detection and isolation of *Listeria* in foods.

Classical methods have used the Christie-Atkins-Munch-Peterson(CAMP) phenomenon to detect hemolysis on blood agar as a tool to distinguish between virulent and avirulent strains of *Listeria* species. However, there are a number of objections to this test including its long test time and mistaken identification of a number of other bacterial species as *Listeria*.

This report presents briefly the state of the art related to this proposal. In contrast to the traditional rapid detection methodology that this proposal contained and in part researched, time sensitive commercial test systems are now use. It is my recommendation to adopt of commercial tool kits rather than those proposed and in part researched by this contract.

1.0. INTRODUCTION

The presence of pathogenic microorganisms in various food products can be a significant health risk to humans. Foodborne illness occurs when a person gets ill by eating food that has been contaminated with an unwanted microorganism. This condition is often called food poisoning and can be due to bacteria, fungi, parasites and viruses. Epidemiological data from developing and industrialized countries suggest that the frequency of foodborne illnesses are unacceptably high. Unfortunately, contamination of a specific food supply by such organisms is not always easy to define. Very often, many food poisoning outbreaks are not reported or dismissed as stomach flu. Some of the most common symptoms of foodborne illness, include stomach cramps, nausea, vomiting, fever and diarrhea.

Although most people are familiar with different bacteria that cause gastroenteritis there are some microorganisms found in food that are pathogenic but do not cause the symptoms associated with gastroenteritis. For example, the mold *Aspergillus flavus* has been isolated from peanut meal and found to produce an aflatoxin that can cause acute liver damage, liver cirrhosis, tumor induction and teratogenesis (Stoloff, 1983; Koshinsky and Khachatourians, 1991; Koshinsky et al., 2000). As well, grains and processed human foods are occasionally contaminated with mycotoxins produced by molds such as those that belong to the genus *Fusarium* (Miller, 1995, Koshinsky et al., 2000). Toxoplasma, a microscopic parasite, has been isolated from fruits and vegetables that have been harvested from gardens contaminated with cat feces and it can cause fevers, enlarged lymph nodes and even sometimes pneumonia. Of the reported foodborne disease outbreaks reported in the United States during an 18-year period the majority of the illnesses were due to unknown etiologic agents. The next most common agents included bacteria with only minor occurrences due to viruses, parasites and toxins.

In order to ensure food safety analysis of food for the presence of pathogenic bacteria should become a required practice. During the past 70 years a number of different methods have been developed for the detection of foodborne microorganisms and their toxins. In most countries statutory requirements of food safety have been established and they are based on the testing of prepared foods for the pathogenic microorganisms and/or toxins (Khachatourians and Arora, 1999). In addition to surveillance testing and monitoring further tests, such as storage tests and microbiological challenge testing were developed. The traditional approach towards controlling food safety involves monitoring and surveillance. This includes inspecting production facilities and operations and microbiological testing of the finished food product.

The International Commission on Microbiological Specification of Foods (1986) has been responsible for establishing the microbiological criteria and numerical limits for various microorganisms in different foods. Storage tests and microbiological challenge testing are sometimes employed to provide further relevant information. For example, storage tests can be undertaken to determine the microbiological consequences in the product after processing, distribution, storage and preparation by the consumer. Microbiological challenge testing involves inoculating the food product with the relevant microorganism. The fate of the organism is then determined after simulating processing, distribution and handling of the food product. Since both of these tests are not routinely run on a continuous basis in the food industry it has not been felt that it is necessary for scientists to develop rapid and automated assays for the undertaking of these tests.

However, within the last decade several advances have taken place in the development of diagnostic assays that are based on such things as immunological properties and nucleic acid sequences of specific genes of the pathogenic food spoilage microorganisms. It is well understood that such advances in rapid detection methods are not a solution to the problems of food contamination, but by increasing the sensitivity and developing time of these assays they have become valuable tools in defining the problem, therefore, changing the practice from on as needed basis to a required for. Monitoring of the finished products may not guarantee safety as sometimes faulty batches escape detection. One thing that is required for all microbiological methods that are to be used in food analysis, whether they are rapid or conventional methods, is quality assurance. Generally, quality assurance requires well-described test methods, the use of reference material and testing in collaborative studies.

Food products for human consumption are routinely inspected for foodborne pathogens but also for commensals that become human pathogens in immunosuppressed hosts. Testing for food contamination is a massive task due to the large amounts of food being distributed throughout the world and the many stages of production to processing and storage it goes through from the livestock operation, distributors, stores and household situation. Thus the production of safe foods is really based on the identification of possible problems before they occur. Frequent testing for pathogens is necessary, as there are many places and occasions where contamination can occur. These tests are performed using conventional bacterial testing methods that are based on the use of selective enrichment and subsequent plating. Although the analytical limit of these methods are very sensitive, they are time consuming and rely specifically on the ability of the microorganisms to grow into visible colonies.

Even today the conventional methods for the detection of *Salmonella* takes 4 days for a negative result and at least 5 days for a positive one (Trkov et al., 1999). In the case of *Listeria*, cold enrichment is often used to take advantage of the ability of this pyschrotroph to outgrow competing microorganisms at low temperatures. Unfortunately, this may require an incubation period of up to several weeks in duration (Faber and Peterkin, 1991). Our experience through the M.Sc. thesis of B. Sampathkumar (1998) and additional work by E. Tsougriani and I.J. Xavier, resulted in an expanded detection methodology. This contribution (Sampathkumar et al., 1999) made use of the production of Listeriolysin O by *Listeria monocytogenes* (Scott A) under stress conditions. Nonetheless, we could not bypass the incubation step that required 4-6 days of waiting. Our quantitative microtiter hemolysis assay for *Listeria monocytogenes*. (Sampathkumar et al., 1998) however, made the identification easier and the results unequivocal. Beyond the element of time and delectability, the ultimate goal for diagnostic research in food microbiology is not only to develop assays that are able to detect microorganisms early, but also have the ability to characterize the isolates. This can be done by employing microbiological, serological, immunological, molecular biological, biochemical or biophysical methods within simple rapid detection kits (Fung, 1995).

Thus the speed of rapid tests could reduce costs associated with delays in moving the food from the producer to the market and since they are small and simple kits no high-cost laboratory equipment is necessary to undertake the tests.

2.0. SCIENTIFIC BACKGROUND

Within the last 10 years immunological and nucleic acid detection based techniques have been developed for use in the food industry to detect a number of different pathogenic microorganisms and toxins. There is now a wide array of assay formats available commercially that use these techniques. These assays range from being very simple such as the latex agglutination assays to very specific DNA amplification methods. There is no doubt that molecular biological assays are more sensitive and specific than conventional microbiological methods and no longer rely solely on culture media to enrich, select and detect the pathogens.

2.1.. IMMUNOLOGICAL ASSAYS

Immunological assays or immunoassays are analytical tests that involve the use of antibodies. These assays can be qualitative or quantitative and take advantage of the specific binding of an antibody to an antigen. Immunoassays can be divided into two groups based on their formats: homogeneous and heterogeneous. In a homogeneous assay it is not necessary to

remove the bound antibody from the unbound and when the antigen-antibody complex is formed it is directly visibly. Examples of such assays are immunodiffusion and agglutination reactions. Homogeneous assays generally involve short reaction times easier to automate than heterogeneous assays and are available for most pathogens (deBoer et al., 1999). In the case of heterogeneous assays the unbound antibody must be removed from the unbound antibody by a labeling reagent. These assays usually consist of four components and include analyte capture, reporter addition, washes and reporter detection. An example of such an assay is the enzyme-linked immunosorbent assay or more commonly known as ELISA.

2.2. DNA BASED ASSAYS

A number of nucleic acid-based assays have been developed over the past few years (deBoer and Beumer, 1999). The use of nucleic acid technology in diagnostics of foodborne pathogens has matured into a dominant technology in the last couple of years. Genetically based methods for the detection of food pathogens can involve nucleic acid hybridization (NAH) or amplification through the polymerase chain reaction (PCR). In both cases genetic detection of foodborne pathogens involves using different regions of the genomic DNA as targets to determine the genus, species and strain-specificity of the microorganism (Scheu et al., 1998).

In the early 1980's DNA probes were introduced for the detection and analysis of foodborne pathogens (Hill, 1981). Nucleic acid hybridization detection systems allow for the discrimination of target organisms from closely related organisms based on characteristic DNA or RNA sequences. The advantage of this approach over a phenotypic approach is that it does not require gene expression by the target organism. In the case of NAH the target DNA or RNA forms a nucleic acid hybrid with a DNA probe that is complementary with the target sequence.

The length of DNA probes are generally limited to 15 to 30 nucleotides and are often referred to as oligonucleotide probes. Although the specificity of the hybridization assay is controlled for the most part by the specific sequence of the probe it is also affected by the stringency of the hybridization buffer. Stringency can be defined as a measure of the degree of base pair mismatch that can be tolerated in the formation of the hybrid and involves the hybridization temperature and salt concentration of the hybridization solution. During hybridization a low stringency can be obtained by decreasing the temperature or increasing the salt concentration. Quite often it is necessary to adjust the stringency as all target sequences may not be identical and a certain tolerance of mismatch will be necessary for detection of all targets. For the detection of detection of virulent *Yersinia enterocolitica,* the M.Sc. thesis of L. R. Jategaonkar (1998) demonstrated that a DNA probe and whole colony hybridization could

generate diagnostic data. Further this work demonstrated that good correlation was present between the probe hybridization and the classical crystal violet binding assay for the detection of this meat associated pathogen.

There are two other methods, bioluminescence and ice crystal formation that can be used to detect the presence of foodborne pathogens.

Bioluminescence is a light-yielding reaction that involves oxygen, ATP (an energy source), a long chain fatty aldehyde and the enzyme luciferase. Ulizer and Kuhn (1987) introduced the idea of identifying bacteria using bioluminescence by inserting *lux* genes from *Vibrio fisheri* into Charion 30, which is a bacteriophage specific for *E. coli*. Bacteriophages do not have the mechanism for expression of the lux genes so they remain non luminescent, however when they infect their host the genes are inserted into the host genome and expressed.

Ice nucleation assay is based on the same principles as bioluminescence, but instead of the *lux* gene it relies on the physical properties of *ina* gene product for ice crystal formation. In a homogeneous environment very small volumes of water can be super cooled to -40^{o}C and still remain a liquid, however, in a heterogeneous environment where nucleating agents are present ice crystals can form at temperatures of -2^{o}C. Certain bacteria such as *Pseudomonas, Xanthomonas* and *Erwinia* synthesize proteins that have the ability to serve as templates to promote ice formation in aqueous solutions by binding the water molecules and simulating the configuration of ice crystals. These proteins are encoded by the *ina* gene (Warren and Wolber, 1991). The Bacterial Ice Nucleation Diagnostic (BIND) has been developed by Idetek (Sunnyvale, California) to assay for the presence of *Salmonella*. In this case, the *ina* gene has been cloned into a genetically engineered phage that is broadly specific for *Salmonella* (Wolber and Green. 1990). If *Salmonella* is present in a food sample, the gene will produce these proteins. By simply lowering the temperature to -9.3^{o}C the formation of ice crystals can then be confirmed by using a freeze-indicator dye. This indicator dye is green and fluorescent , but when the medium is frozen it becomes orange and non fluorescent (Wolber and Green, 1990). The BIND assay has been determined to have a detection limit as low as 20 CFU/ml, but generally it ranges between 100 and 10,000, and pre-enrichment is still required for optimal detection (Wolber et al., 1995).

In the BIND ice nucleation assay an aliquot of the enriched sample is treated with the phage and then incubated for 2.5 hours to allow for phage infection and transcription/translation of the ina genes. After a 20-minute supercooling period the sample can be checked for color changes.

3.0. COMMERCIAL KITS

During the late 1980's few scientists would have predicted that the basic molecular biological methods involving antibodies and nucleic acids would dominate the field of food diagnostics (reviewed Feng, 1997). In the field today there are a number of assays available commercially that incorporates these technologies for the detection of specific foodborne pathogens and toxins. There are numerous companies that produce and sell various assays' kits for the detection of foodborne pathogens. Although kits are available for detecting a wide variety of pathogenic organisms this review will focus specifically on those that are able to detect *E. coli* (specifically O157), *Salmonella* species and *Listeria* species.

3.1. COMMERCIALLY AVAILABLE ANTIBODY-BASED ASSAY KITS

There are numerous antibody-based assay kits commercially available for detecting *E. coli* O157, *Salmonella* and *Listeria*. Majority of these assay kits are based on latex agglutination, ELSIA or antibody precipitation. Several different companies produce rapid test kits that involve latex agglutination. For example, REMEL, PRO-LAB, Orion Diagnostica and Unipath manufacture kits specifically for detecting *E. coli* O157 in food samples. The RIM *E. coli* O157:H7 Latex Test produced by REMEL can be used for the detection of both *E. coli* O157 and H7 antigens. It consists of O157 latex, H7 latex, control latex as well as positive and negative controls. Wampole Labs, Rhone-Poulenc, REMEL, Unipath and Mercia also produce latex agglutination kits that are specific for detecting *Salmonella*. At the present time, however, the latex agglutination format is not readily used to detect *Listeria*.

Several other companies manufacture commercial kits based on enzyme-linked immunosorbent assays. BioControl Systems Inc. of Bellevue, Washington manufactures the Assurance EIA kit that is for the detection of not only *E. coli* O157, but also *Salmonella* and *Listeria*. International BioProducts produces the TECRA system for the detection of these organisms. This system is a visual immunoassay that in the case of *Salmonella* uses affinity purified polyclonal antibodies. It can be performed immediately following enrichment and results are obtained within one hour. Positive results are indicated by the development of a green color. Diffchamb offers two products for the detection of *E. coli* and *Salmonella*. The Transia Card is a rapid ELISA test that is based on a one-step immunoassay reaction. In the case of *E. coli* this device has a dye pad that is impregnated with anti-*E. coli* O157 antibody-dye conjugate. Once the sample is added to the sample well the *E. coli* O157 antigens will react with the conjugate dye and bind to the antibodies immobilized in the test window, which will result in the formation of a colored band. The Transia Plate was developed to enhance the

sensitivity of the Transia Card. This test is based on a sandwich-type immunoenzymatic reaction that uses, in the case of *Salmonella*, a mixture of antibodies to ensure the detection of *Salmonella* in the sample. Both of these assay kits can be used as test matrices for meat, poultry, eggs, other food products, feedstuff and environmental samples.

The third assay format commonly employed in commercial detection kits involves antibody-precipitation. Some of the kits based on this format that are currently available include VIP (BioControl), Reveal (Neogen), NOW (Binax), Clearview (Unipath and PATH-STIK (LUMAC). The VIP assay can be used to detect *E. coli* O157, *Listeria* and motile and non-motile *Salmonella*. This is a very simple assay that is made active by adding the enriched sample to the sample addition well. The flow then begins laterally from this well and across the reagent interface zone. If the target antigen is present in the sample then it will react with reagents contained in the device to form an antigen-antibody chromogen complex. The flow continues across the detection zone where the antibody-antigen complex will react with the proprietary reagents. Following a few minutes of incubation at room temperature a line forms in the first window indicating a positive test. The flow then continues through the verification zone and at this point all samples, whether the antibody-antigen complex is present or not, reacts with reagents to form a line and confirm the test has worked and is complete.

Although most of the antibody-based detection kits available incorporate the assay formats of either latex agglutination, ELISA or antibody-precipitation there are some kits available that are based on different formats. For example, Dynal produces the kits known as Dynabeads, which can detect the presence of *E. coli* O157, and *Salmonella*. VICAM also produces antibody bead kits known as Screen and Listertest that are used to detect *Salmonella* and *Listeria*, respectively. Other formats include antibody blots, tube-EIA, sera and diffusion.

3..2. COMMERCIALLY AVAILABLE ASSAY KITS BASED ON PCR

Within the last decade traditional and immunological methods for the detection of foodborne pathogens have been supplemented by a number of DNA-based methods. The main focus of DNA-based methods has been on the in vitro amplification methods, which include nucleic acid sequence based amplification (NASBA), Q beta replicase amplification, LCR and PCR. However, except for PCR the other methods have been found to have only limited practical relevance in detecting foodborne pathogens. This is not due to lack of research on the applications of these procedures. As mentioned earlier there are other research data on the significance of the detection and discrimination of *Listeria* species (Wiedmann et al., 1992).

Within the past few years several companies have developed PCR diagnostic kits for the detection of *E. coli* O157, *Salmonella* and *Listeria*. In order to develop kits, that are based on PCR technology, that are sensitive and accurate it is necessary to determine a region for amplification that are unique to the bacteria of interest and develop a detection system. In the case of *E. coli* O157 the gene regions used for amplification are the verotoxin 1 and 2 genes *slt* I and *slt* II, respectively. Several different regions can be used for the detection of *L. monocytogenes*. These include *prf* A gene, 16SrRNA, *hly* A gene, listeriolysin O-gene, *iap* gene, hemolysin gene and the *dth* 18-gene. The detection system used to test for the presence of the specific amplified product varies depending on the kit. The products can be identified by running the PCR products on an ethidium stained agarose gel or by using sophisticated equipment such as the Perkin-Elmer/Applied Biosystems Sequence Detector. This system basically detects the amount of a fluorescent signal that is produced proportionally during the amplification of a PCR product.

Evidently while the number of commercially available foodborne pathogen detection kits using PCR at this time is very small the future trend will be the opposite. Qualicon manufactures a PCR kit known as the BAX system, which detects *E. coli* O157, *Listeria* and *Salmonella*. These kits have been simplified for use by combining all the requisite primers; polymerase and nucleotides into a single tablet that is prepackaged into the samples' tubes. BioControl produces the Probelia PCR system for *Salmonella* and *Listeria*. The test components of these kits consist of PCR for amplification and micro plates for detection. In this case a colorimetric microplate assay is performed on the amplified DNA products and the results read using a microplate reader. PanVera also manufactures detection kits for *E. coli* O157 and *Salmonella*. Similar to other kits PanVera includes a premixed solution containing all the reagents required for PCR.

In September 1996 Perkin-Elmer introduced Taq-Man, the first automated DNA-based test for the detection of *Salmonella*. The Taq-Man PCR amplification/detection kit is a gel-free kit that is based on the fluorogenic 5' nuclease assay and takes advantage of ABI PRISM multicolor fluorescence labeling technology. This involves the use of highly specific primers and a fluorescently labeled TaqMan probe which is complementary to a conserved region on the genome. In these kits the target organisms are detected by monitoring changes in the fluorescence emission from the probes using the ABI PRISM Sequence Detection System.

4.0. EVALUATION OF CONVENTIONAL AND RAPID METHODS

Traditionally methods to detect foodborne pathogens have relied on conventional ones that involve selecting and propagating viable cells from foods on specific culture media. With the advances that have occurred recently in biotechnology the power of DNA analysis and immunology have been incorporated into the discipline of detecting foodborne pathogens. Currently, however, many of the assays available are only used for screening purpose and all positive results are still confirmed using conventional methods in order to comply with government regulations and receive Canadian Food Inspection Agency (CFIA) and United States, Food and Drug Administration (FDA), approval. All new assays developed for identifying pathogens or toxins must undergo vigorous testing and be compared thoroughly with standard microbiological methods prior to routine use in food analysis. This involves the use of a number of validation and evaluation programs and in the USA the most widely accepted is AOAC International Official Method program. These methods have been written into government regulations, product acceptance criteria, product specifications and are relied upon in legal proceedings. Thus, by tightly regulating the food industry there will be fewer lawsuits and malpractice suits due to inadequate testing and the consumer will have more confidence in food products available.

5.0. FACTORS TO CONSIDER IN RAPID ASSAYS

5.1. ENRICHMENT AND SELECTION

Presently, both rapid immunological- and PCR-based assays require an enrichment procedure prior to the assays being undertaken. Enrichment is an essential component to successfully confirming the presence of pathogens, such as *Salmonella* spp., *E. coli* and *Shigella* spp., since food is composed of several different ingredients, some of which can adversely affect the viability of bacteria. This becomes a very important factor when one considers that pathogens such as *E. coli* O157 and Shigella have very low infectious dosages and without proper enrichment both types of rapid assays may not successfully identify their presence.

Currently the enrichment procedures used in food microbiology consist of two distinct steps. The first step, which includes pre-enrichment, allows for the normal flora and target microorganisms to multiply until no further growth occurs. The second step involves removing the sample and incubating it in a selective broth, which acts to inhibit the growth of competing microorganisms and allow for the pathogen to grow to detectable levels. It is these steps, which are essential, that are the time-consuming factors in detecting foodborne pathogens. Once the

enriched sample has been obtained both the immunological and PCR-based assays can give results within a few hours.

5.2. ACCURACY, AUTOMATION, MATRICES, AND SIMPLICITY

In developing and adapting rapid assays there are several factors that food scientists must take into consideration. These factors include accuracy, speed, automation, sample matrix, simplicity and cost to the company (Fung, 1995). Accuracy is probably the most important factor that has to be taken into consideration since we are dealing with microorganisms that cause severe illnesses at low dosage levels. Thus it is essential that the rapid test systems being used are accurate and false-negative or false-positive results approach zero. For safety reasons false-negative results can not be tolerated. Another important factor to take into consideration is the amount of time it takes to obtain accurate results. The results of rapid test should be within a day and no longer. However, as previously mentioned rapid tests, like conventional methods, require a period of enrichment that can be quite time consuming.

Automation allows for a large number of samples to be tested at the same time. For example, many of immunoassays currently available use microplate format that can handle up to 96 samples at one time. Companies have also developed single-use kits for the opposite scenario where only a few samples must be run at one time.

Matrices in any food sample differs. Matrices are important in that newer assay systems perform compatibly and well with a particular matrix under examination. Presently PCR inhibition due to a particular food matrix is overcome by separating the bacteria from the matrix prior to DNA extraction.

Finally, simplicity of the assays and cost of running the tests to the company are two factors that must be taken into consideration when developing rapid assays. Due to the fact that people with different levels of training may be working in one lab it is necessary that the assays be user-friendly. The easier the kits are to use the less likely mistakes will arise. It is also essential that training, if required, and technical service be available to the food laboratory. The final cost to the end user or company is a very important factor. As well, one must consider not only ongoing purchase of the assay kits, but also that of reagents, operations and maintenance. Generally, immunoassay and DNA-based assay kits are quite expensive with costs ranging from $350.00 to $500.00 US. As well the initial financial investment may be quite high especially in the case of DNA-based assay kits as thermocyclers are absolutely required. Alternately, region specific testing centers could be developed that are

jointly supported by government and industry. In its absence out source, contracts with local University Laboratories could be another option.

6.0 CONCLUSIONS

Today, detection of foodborne pathogens relies less on standard cultural methods because there have been significant improvements in the fields of immunology and molecular biology. These improvements have led to the development of several different types of rapid assay kits. However, despite better detection efficiencies these rapid assays are still affected by the food matrix being tested, food ingredients and normal flora. Rapid assays, like cultural methods, also require an enrichment step that can be time consuming. Due to these reasons as well as others, most companies that are involved in detecting foodborne pathogens only use rapid assay for screening purposes and all positive results are still confirmed by standard cultural methods.

In order to comply with government regulations and guidelines the food industry must rely on conventional microbiological methods that have CFIA and FDA acceptance to evaluate all stages of food processing. Further research needs to be undertaken to expedite methods for the detection of foodborne pathogens. However, the ultimate goal in food microbiology has to be to develop assays that have the ability to characterize isolates at sensitivity levels that would allow for CFIA/FDA approval. By developing such assays it may be possible to reduce costs with delays in moving the food product to the market and lower the costs of setting up food testing laboratories.

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8.0. ACKNOWLEDGEMENT

The work conducted leading to and including this report was supported by Saskatchewan Department of Agriculture and Food. We appreciate the role that the Department through Agriculture Development Fund program plays in the support of agri-food research.